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Testosterone: More Than Having the Guts to Win the Tour de France

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Sex bias in susceptibility to autoimmune diseases is evident but poorly characterized. Yurkovetskiy et al. (2013) report that host testosterone mediates changes in the microbiome to confer protection to adult male NOD mice from type 1 diabetes.

Autoimmune diseases are disorders where inappropriate immune responses are directed toward self-antigens and result in destruction of self-tissues. It has long been recognized, both in humans and in experimental animal models, that females are affected more than males by an array of autoimmune diseases (Whitacre, 2001). Several explanations for this observation have traditionally been given, including different basic immune responses in females, direct and indirect modulation of the immune system by sex hormones, and genetic factors contributing to the susceptibility of autoimmune disorders (Pennell et al., 2012). However, the issue is clearly more complex than these explanations—alone or in combination—can account for. For example, it is well known that concordance of autoimmune diseases is low in homozygotic twin pairs. This low concordance rate argues for the importance of environmental and epigenetic factors that are not encoded in the genome but are critical for the initiation and progression of these disorders (Mathis and Benoist, 2012). Research is beginning to unravel the exact nature of these factors.

A breakthrough in the search for additional causes of sexual predisposition to many autoimmune diseases came when Markle et al. (2013) revealed that sex differences in mice result in unique gut microbiomes and that these differences in the microbiome can drive the sex bias in autoimmunity in a hormonally dependent pathway. These findings, together with those of other investigators, strongly support the idea that the microbiota, as an environmental and epigenetic factor, can actively influence host physiology and impact host disease susceptibility (Chung et al., 2012; Olszak et al., 2012).

In this issue of *Immunity*, Yurkovetskiy et al. (2013) provide new evidence offering remarkable insights into the mechanism underlying what appears to be dual regulation of sex-biased type 1 diabetes (T1D) by androgens and the microbiota (Figure 1). By retrospectively analyzing literature reports on the occurrence of T1D in nonobese diabetic (NOD) mice, the authors show that male-to-female incidence ratios for T1D vary widely in NOD mice from facility to facility and even as a function of time. In contrast, germ-free male and female NOD mice always are similarly susceptible to T1D. The researchers hypothesized that the compositions of the male and female microbiotas, which differ over time and between facilities, influence the sex bias toward development of T1D in NOD mice. To directly address this hypothesis, Yurkovetskiy et al. analyzed the microbiota of NOD mice of both sexes before and after puberty. They found that female adult mice have microbiomes similar to those of prepubescent mice of both sexes; however, the commensal microbial community in adult male mice significantly deviates from this shared initial pool. Strikingly, the authors show that the microbiome in castrated adult males clearly shifts away from that of normal adult males and is closer to the microbiome of females. The incidence of T1D in these mice is positively correlated with the "femaleness" of the microbiota. Moreover, they demonstrate that microbiota-mediated sex-biased protection in males is independent of MyD88. These results support the hypothesis that the host androgen level is influential in determining the composition of the microbiota, which in turn affects T1D initiation and progression. It will be important to investigate whether androgen-treated adult female mice can support a male-like microbiota and thus be protected from T1D.

Next, Yurkovetskiy et al. sought to determine what microbes are uniquely present in the microbiomes of male and female NOD mice and which of these microbes are relevant to sex bias in T1D. In four independent experiments, the authors found no universal unique "male microbiome"; however, they did find that four distinct combinations of microbial groupings (with an interesting lack of overlap at the individual family level in the four experiments) were enhanced by androgen based upon the input microbial community. It would have been informative had similar sex-biased T1D incidence been verified in these four groups of mice. By using gnotobiotic techniques, the investigators found that only certain microbial species enriched in adult males confer protection from T1D and mediate the sex bias in gnotobiotic NOD mice. The two verified species are quite distinct phylogenetically: one species consists of the segmented filamentous bacteria (SFB) and belongs to the Firmicutes, whereas the other is an *Escherichia coli* or *Shigella*–like (SECS) strain belonging to the Proteobacteria. These results, along with the fact that no universal microbial groups were identified, suggest that a core set of microbial properties, functions, or genes, working in concert with testosterone, is essential for testosterone-conferred protection against T1D. It will be important to analyze the genome-encoded metabolic pathways of the four different sets of microbes enriched in adult males. It is possible that, despite taxon diversity, these sets share a conserved metabolic signature that is

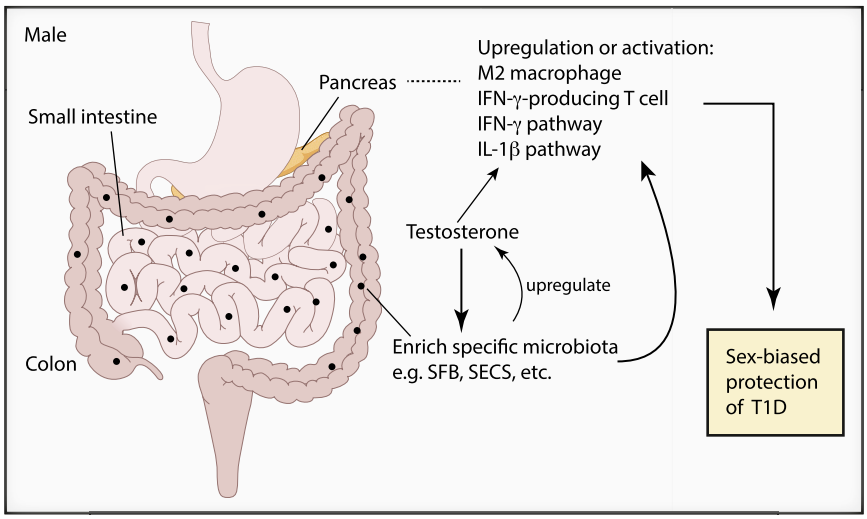

Figure 1. Testosterone and Testosterone-Specific Microbes Lead to Sex-Biased Protection in T1D

The incidence of T1D is significantly higher in female compared to male NOD mice. In the adult NOD male mouse, a high testosterone level enriches the microbiota for specific organisms such as segmented filamentous bacteria (SFB) and *Escherichia coli* or *Shigella*–like (SECS) strains. These microbes also upregulate host testosterone. A minimum level of testosterone and specific male-enriched microbes working together upregulate M2 macrophage and IFN-γ producing T cells in pancreatic lymph nodes. Microarray data show that both the IFN-γ and IL-1β pathways are also stimulated. Yurkovetskiy et al. propose that these pathways lead to a sex-biased protection of T1D.

important for the mechanism of interest (Human Microbiome Project Consortium, 2012). Furthermore, the genomes of SFB and SECS can be scrutinized by means of microarrays and RNA sequencing as alternative approaches to gaining insight into the "teaming up" of microbial functions with testosterone.

Yurkovetskiy et al. asked whether the "male microbiomes" can impact host testosterone levels. They found that colonization with protective microbiomes—e.g., SPF microbiota, SFB, and SECS—is positively correlated with high blood testosterone levels in male mice. However, beyond a concentration of 2 ng/ml, more testosterone does not confer extra benefit. Intriguingly, a high testosterone level alone does not predict protection; a defined "complete" flora, designated the altered Schaedler flora, does not protect NOD males from T1D despite maintenance of the host's blood testosterone concentration at ~5 ng/ml. Further complicating the issue is the observation that SPF males have lower testosterone levels than germ-free males. These results suggest that, in addition to testosterone and certain microbes, other unidentified factors are involved in influencing sex-biased protection.

Describing the results of studies designed to reveal how testosterone and microbes protect against T1D, the authors report that certain host signaling networks, including the interferon-γ (IFN-γ) and interleukin-1β (IL-1β) pathways, are specifically and differentially expressed in the pancreatic lymph nodes (PLNs) of male mice due to the presence of microbiota. They found that M2 macrophages are more abundant in SPF males and are critical for the observed gene-expression signatures in these mice. In addition, there was a higher percentage of IFN-γ-producing T cells in PLNs of male mice than in PLNs of female and castrated male mice. Finally, they confirmed that, after exposure to SECS, peritoneal macrophages from adult males are more efficient in eliciting IFN-γ from insulin-specific $CD8^+$ T cells than are those from females. These experiments call for elucidation of mechanisms that could paradoxically link the M2 macrophage and the activation of the IFN-γ pathway in T cells. Another appealing study would be to determine whether these same genes are upregulated when adult males are colonized with each of the four different microbial combinations identified. It will also be interesting to investigate whether similar M2 macrophage activity and IFN-γ production are involved in protection in other T1D disease models.

A direct implication of this study is that probiotic administration or fecal transplantation is a theoretically possible approach to protection against T1D. However, such an approach is impractical because of the inability to prospectively identify people at risk. It is also unclear how long the conferred protection would last because the microbiota is highly host-specific (Chung et al., 2012) and androgen is probably required continually to retain the transplanted male signatures of the microbiome. Another more fundamental approach is to identify the detailed pathways and molecules from both host and microbes that mediate protection and to translate this knowledge into the designing of new therapeutics. This approach is very promising but also challenging. So far, just a handful of microbes, including *Bacteroides fragilis* and SFB, have known molecular interactions with the host immune system (Chow et al., 2010). Nonetheless, this study and the earlier report by Markle et al. (2013) have established a new direction for treating autoimmune diseases.

Intestinal microbes are immersed in a complex host chemical and biological environment that not only provides a nutrient-rich niche but also imposes tremendous selection pressure on the microbiota (An et al., 2011). How specific host factors and the microbiota shape each other and together impact health and disease is a question that has attracted increasing interest in the past decade. The work by Yurkovetskiy et al. is an important contribution to elucidation of the role of interplay between sex hormones and the microbiota in disease susceptibility. Only a few other types of hormones—for example, those involved in pregnancy and stress-response—have been suggested as having a possible role in regulation of the microbiome. Indeed, as critical messengers delivering signals from one cell to another, it is not surprising that hormones might mediate direct conversations between host cells and symbiotic microbes, which have coevolved for eons. Insights from these conversations will undoubtedly enhance the quality of health care.

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